

Bone Marrow: Indications and Procedure Set-up

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Abstract

The bone marrow is a complex organ, with dynamic hematopoietic and immunologic functions. It is disseminated within the intertrabecular and medullary spaces of the bone. Bone marrow trephine biopsy should be carried out by trained individuals following a standard operating procedure. A bone marrow aspirate should be performed as part of the same procedure. Because diagnostic samples are small representation of the total marrow, it is important that the material should be adequate, representative, and of high technical quality.

We present a general review of bone marrow examination, indications and procedure set-up.

Keywords: Bone marrow, trephine biopsy, aspirate, indications, procedure set-up.

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Role of the Bone Marrow Examination

The role of the Bone Marrow (BM) in hematopoiesis was described by Neumann¹ in 1868. Later on, the methods for BM procedure have vastly improved. Currently, indications for pathologic assessment have broadened to encompass a wide range of benign and malignant conditions. Before the paper by Turkel and Bethell² in 1943, examination of a BM aspirate was considered adequate to diagnose hematologic disease. Following the development of newer techniques and equipment,³⁻⁶ BM biopsy became an integral part of BM analysis, and the results of light microscopy are now complimented by cytochemistry,⁷ immunohistochemistry,⁸⁻¹⁰ flow cytometry,¹¹ cytogenetics,¹² molecular genetics,¹³ electron microscopy,¹⁴ tissue culture, and the evaluation of the effects of specific growth factors.⁵ Not all

of these ancillary methodologies are required in every case. In fact, in the hands of a skilled morphologist, a well-prepared aspirate, core biopsy specimen, and iron stain are often adequate to answer many basic questions. Therefore, to ensure accuracy and quality with minimal cost, correlation of the clinical findings with the Complete Blood Count (CBC) and appearance of the Peripheral Blood (BP) smear should precede BM procedure. Such foresight ensures that all samples required for a given situation are obtained during the initial procedure with minimal patient discomfort.

Indications for Bone Marrow Analysis

- To explain a decrease in the formed elements of the blood such as in anemia, leukopenia, thrombocytopenia, and myelodysplasia, and for the determination of bone marrow cellularity.

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- To explain an increase in the formed elements of the blood such as in unexplained sustained leukocytosis, thrombocytosis, myeloproliferative disorders, and leukemias at time of initial diagnosis, and to monitor the progress of therapy.
- To explain abnormal morphologic changes observed in the peripheral blood, i.e., tear drop erythrocytes, rouleaux, immaturity of myeloid cells, lymphocytes, and leukoerythroblastosis.
- To assess the adequacy and location of stainable BM iron.
- To evaluate BM involvement by metastatic disease, malignant lymphoma, plasma cell dyscrasia, myelofibrosis, and mast cell dyscrasia.
- To obtain cellular BM for cytochemistry, flow cytometry, cytogenetics, molecular genetics, immunohistochemistry, microbial cultures, electron microscopy, and tissue culture.
- As part of the workup for fever of unknown origin, to assess for bone marrow involvement in infections, in granulomatous disorders, and in patients with unexplained adenopathy and hepatosplenomegaly.
- To evaluate for BM involvement in suspected storage diseases and collagen vascular disorders.
- To assess unexplained osteosclerosis and other abnormalities of trabecular bone detected by radiologic studies.

Bone Marrow Procedure and Specimen Components

Because of differences in organizational structure, patterns of reimbursement, and personal philosophies of management, policies governing bone marrow procedure vary from one medical facility to the next. Centers with an organized procedure room, trained support services, and defined policies for specimen acquisition are usually best equipped to provide quality services to all departments and consistently retrieve optimum diagnostic material. For the convenience of patients and medical personnel, the BM procedure room is ideally located in the hematology-oncology clinic. Additionally, a well-equipped bone marrow cart is invaluable to support acquisition

in operating rooms and wards and from non-ambulatory patients, such an arrangement is also recommended for office practices from which specimens must be transported elsewhere for processing. Knowledge of the clinical history, CBC, and appearance of the PB smear before procedure invariably serves to define which components of the bone marrow will be most useful to establish a diagnosis.¹⁶

To maximize information, evaluation of both the aspirate and the core are recommended as standard procedure in adults,¹⁷⁻²⁰ in infants and children, an aspirate may be the only sample submitted for interpretation. Both components are unique and complimentary in their ability to provide specific information. The most frequently used site is the posterior superior spine of the iliac crests, and this provides access to both the aspirate and the core. The former may also be obtained by sternal puncture. This site should never be used to obtain a core biopsy specimen.

The Range of tests that can be performed on bone marrow aspiration material is outlined in Table (1). An optimum aspirate^{21,22} of about 3.0mL ensures that 6-8 aspirate smears can be prepared, thus providing adequate material if cytochemical studies and analysis for Terminal Deoxynucleotidyl Transferase (TdT) are indicated. A minimum of two smears should be stained with Wright-Giemsa and one with Prussian Blue for evaluation of iron stores. Appropriate identification of BM particles (spicules) from the aspirated material ensures adequate cellular representation in smears. This is best achieved by separating sinusoidal blood from bone marrow particles in a tilted Petri dish containing the freshly drawn aspirate. Particles adhere to the Petri dish and are easily lifted off with a pipette. In situations where smears cannot be prepared from the aspirate immediately, or where they need to be transported to a laboratory, the sample should be anticoagulated in a purple-top (EDTA) tube at room temperature. Buffy coat preparations of anticoagulated sinusoidal blood from aspirate samples without particles (i.e., dry tap) usually result in a concentration of BM elements and facilitate interpretation.

Table (1): Range of Tests Performed on Bone Marrow Aspiration Material.

Smears

Wright-Giemsa stain
Prussian Blue stain
Cytochemistries and TdT
Microbial stains

Touch prep of clot

Wright-Giemsa stain

Paraffin section of clot

H/E and Prussian Blue stains
Immunohistochemistry and molecular diagnostics

Flow cytometry

Cytogenetics

Molecular diagnostics

Microbial cultures

Electron microscopy

Tissue culture

TdT = terminal deoxynucleotidyl transferase; H/E = hematoxylin and eosin.

The choice between crush, wedge, cover-slip, and slide preparations of the aspirate is often personal; each works equally well in the hands of experienced professionals. In our opinion, material that is cover-slipped has an advantage. Cover-slipping improves the refractive index and provides a wider visual field when scanning the slide at low and high power, ensuring shorter review times and greater ease in selecting areas that require examination under oil. Additionally, the cover-slipped slide ensures against loss of tissue during cleaning and filing.

Preview of a Wright-Giemsa-stained aspirate smear immediately after acquisition is of considerable value in the triage of the aspirated sample, and can be an important cost-conserving measure. Smears for TdT (using immunofluorescence or immunoperoxidase) should be kept in a desiccator for optimal results. For all other special procedures, smears may be air-dried and retained at room temperature. In cases requiring microbial stains, when a report is required on the same day as the procedure (as in patients with acquired immunodeficiency syndrome with low CD4 counts), or when the core and particle sections are inadequate, these stains may be performed on aspirate smears.

As part of the routine diagnostic work-up, microscopic examination of the bone marrow aspirate provides an opportunity to enumerate its

cellular components. These are preferably reported in a 500-nucleated-cell count excluding osteoblasts, osteoclasts, fat cells, stromal cells, macrophages, endothelial cells, and tumor cells. Any qualitative nuclear and cytoplasmic abnormalities encountered are also documented.

Preparation of an optimum low volume clot from residual aspirated bone marrow is best achieved by centering an aliquot of aspirate on filter paper with the end of a glass slide. This ensures absorption of sinusoidal blood and concentration of particles. Touch preps of the clot secure cellular material in cases where the number of aspirate smears may be too few to perform a full battery of cytochemical stains. Additionally, Wright-Giemsa stained slides of the touch prep may reveal metastatic tumor a day before paraffin sections become available.¹⁶ Paraffin sections of the clot provide architectural information second only to the core biopsy, enable assessment of iron stores without the effects of decalcification, and serve as a source of cellular material for immunohistochemistry in cases where the core biopsy specimen is inadequate. Aspirates received in anticoagulant do not readily clot. From such samples, particles may be readily secured either by filtration through a mesh or porous histoprep biopsy bag before fixation.

For special studies, aliquots of BM aspirate are submitted as follows:

- Flow cytometry: 2-5 mL. of aspirate in a purple (EDTA) or yellow (ACD) top tube.
- Routine cytogenetics: 2-5 mL. of aspirate in a green (sodium heparin) top tube.
- Molecular diagnosis: samples for molecular assays using Southern blot, Polymerase Chain Reaction (PCR), and fluorescence in situ hybridization (FISH) techniques may be obtained from purple or yellow top tubes.
- Microbial cultures: for aerobic and anaerobic organisms, the BACTEC system (Becton-Dickinson; Sparks, MD) is recommended. These manufacturers also market a MYCO/F Lytic medium in which aspirate samples for acid fast bacilli and fungus may be submitted to the laboratory for primary isolation.
- Electron microscopy: several bone marrow particles free of sinusoidal blood should be fixed in 2% glutaraldehyde or Carson's fixative.

Information obtained from these special methodologies serves to support the findings obtained from proceeding morphologic and cytochemical studies. Profiling of aspirated bone marrow in automated and laser-based systems^{23, 24} has been promising as a screening mechanism, and may in the future reduce the number of cases requiring morphologic assessment.

The range of testing that can be performed on the core biopsy specimen²⁵ is outlined in Table (2). Ideally, the core biopsy specimen should measure at least 20mm in length after processing and if it is not, consideration should be given to repeat the procedure possibly on the contra lateral side. A minimum requirement for evaluation is a hematoxylin-eosin stained section. However, based on the case in question, additional special stains, immunohistochemical preparations and molecular diagnostic studies can provide extra information. Touch preps of the core are of comparable usefulness to those of the clot. The retrieval of diagnostic cellular material for flow cytometry immunophenotypic studies on core specimens in cases with a dry tap has recently been facilitated by vortex disaggregation in RPMI (a culture media developed at Roswell Park Memorial Institute, hence the acronym).²⁶

Such manipulation does not result in structural disruption in postfixed paraffin sections of the core biopsy specimen.

Table (2): Range of Tests that Can be Performed on the Bone Marrow Core Biopsy Specimen.

Paraffin section

H/E stain

PAS stain

Reticulin and trichrome stains

Microbial stains

Immunohistochemical stains

Other special stains

Molecular diagnostics

Touch prep

Wright-Giemsa stain

Immunohistochemical stains and TdT

Vortex disaggregation

Flow cytometry of disaggregated cells

Postfixation and paraffin section of core

H/E = hematoxylin and eosin; PAS = periodic acid-Schiff; TdT terminal deoxynucleotidyl transferase.

The core biopsy specimen provides valuable quantitative information, including the fat:cell ratio, and an overall picture of the BM in situ. Granulomatous and fibrosing lesions are best evaluated in the core. Specific immunohistochemical identification of hematopoietic, plasma cell and lymphoreticular cell lines is now possible,³⁰ making this technology highly complimentary to cytochemistry and flow cytometry. Evaluation of the core is invaluable in patients with bone marrow involvement by Hodgkin disease, where Reed-Sternberg cells are often inaspirable because of the accompanying fibrosis. Additionally, patients with follicular lymphoma with a negative aspirate may have paratrabecular lymphoid aggregates as the sole manifestation of disease. It is now the recommendation of many specialty groups that patients with Hodgkin disease, non-Hodgkin lymphoma (with the exception of lymphoblastic lymphoma, Burkitt lymphoma and undifferentiated non-Burkitt) and metastatic carcinoma be staged with bilateral core biopsies.¹⁶ This is also the recommendation of some observers for patients being worked up for multiple myeloma because this disease can be focal.

Quantitation of stainable iron in the BM should be routinely performed, and is best evaluated from Prussian Blue stains of the aspirate and clot section. Stains of the biopsy also can be done, but may underestimate iron because decalcification can leach out iron stores.

Evaluation of bone marrow reticulin with a Wider's stain or modification of the same is considered standard procedure at some centers. Because reticulin proliferation reflects early fibrosis, such staining can be recommended as part of the general work-up.

Recently, the World Health Organization Classification of tumors of haematopoietic and lymphoid tissues recommended manual 200-cell leukocyte differential of PB smears when the white cell count permits, and a count of 500 nucleated BM cells on cellular smears, preferably around the spicules, as a prerequisite for classification of myeloid neoplasm.³¹

Bone Marrow Artifacts

Finally, types of artifacts that may be encountered in aspirate smears and biopsy sections include those caused by sampling errors, poor technique at the bed side, and poorly performed laboratory procedures all are listed in Table (3).³²⁻³⁴

Table (3): Causes of bone marrow artifacts.

- ✓ *Overstaining or understaining of smears*
- ✓ *Water contamination of staining solution*
- ✓ *Aspirate smears too thick*
- ✓ *Excessive pressure for particle crush preparations*
- ✓ *Small clots on aspirate smears*
- ✓ *Aspiration of marrow from core biopsy sample*
- ✓ *Core biopsy of previous biopsy site*
- ✓ *Core biopsy consisting of hypocellular subcortical region*
- ✓ *Crushed core biopsy specimen*
- ✓ *Inadequate fixation of clot and biopsy specimens*
- ✓ *Suboptimal sectioning and staining of clot and biopsy specimens*
- ✓ *Excessive decalcification of bone marrow biopsy*
- ✓ *Excessive exposure of biopsy to mercurial fixative*
- ✓ *Failure to adequately wash B5-fixed specimens*

Fixation of the core in Zenker's fixative²⁷ with glacial acidic acid provides concurrent decalcification, and ensures a mordanting effect in Giemsa preparations. A formic acid-formalin mixture (Surgipath Decalcifier 1) (Surgipath Medical Industries; Richmond, IL) is another excellent fixative/decalcification agent, and is associated with minimal leaching of iron. An equally acceptable alternative is fixation of the core in 10% formalin, postfixation in B-5 and decalcification in 10% aqueous nitric acid. However, metallic fixatives such as Zenker's and B-5 limit further diagnostic testing by PCR. Zinc-formalin fixatives such as B-plus Fix (BBC; Stanwood, WA) are not associated with this limitation. Decalcification in nitric and hydrochloric acid has been reported to diminish the acid fastness of mycobacteria,²⁸⁻²⁹ and can result in false negatives. Acid fastness is retained following decalcification in formic acid-sodium citrate and citric acid buffer,²⁹ and therefore these are decalcifying agents of choice in cases when infection by mycobacteria is suspected.

Conclusion

Laboratories should have a written, signed and dated procedure for bone marrow aspiration and trephine biopsy. All operators who carry out this procedure must be trained and the procedures they perform must be supervised until competence is assured.

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نخاع العظم: الدواعي والإجراء

رائدة العودات

الملخص

النخاع العظمي جهاز معقد بمهام ديناميكية لها علاقة بتكوين نسيج الدم والوظائف المناعية. يتوزع نخاع العظم في فراغات العظم الداخلية والنخاع. يتم أخذ عينة نخاع العظم من قبل أشخاص متمرسين ومدربين ووفق إجراءات مبينة بمعايير ثابتة وواضحة. وفي العادة يتم أخذ عينة سائل نخاع العظم مع عينة الخزعة في الإجراء نفسه. ولأن العينات التشخيصية هي تمثيل صغير للنخاع فمن المهم أن تكون العينة المأخوذة كافية وذات جودة عالية. يتم فحص العينة والإبلاغ عنها بطريقة نظامية مع مراعاة تقييم الأوعية الدموية، العظم، الستروما، والنسيج المكون للدم وأي نوع من الأنسجة اللمفاوية وغير اللمفاوية.

يجب إصدار نتيجة التقرير من قبل الطبيب المختص بحيث يكون قادرا على تكوين تقييم شامل لكل من السائل والخزعة. كما ينبغي أن يتضمن التقرير وصفا للنتائج النسيجية مع إعطاء تفسير لأهميتها. يجب مراعاة توثيق وتوقيع وحوسبة التقرير ضمن وقت محدد. تقدم في هذه المراجعة استعراضا عاما لكيفية فحص نخاع العظم، الدواعي اللازمة للفحص، وطريقة الإجراء، والمبادئ التوجيهية لتفسير وتكامل المعلومات بحسب سلاله الخلية، وحيز النسيج، والحالة المرضية.

الكلمات الدالة: نخاع العظم، الخزعة، سائل نخاع العظم، الدواعي، الإجراء.